An Integrated Study of the Factors Influencing the Choice of the Settling Site of *Balanus crenatus* Cyprid Larvae[1]

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Settled larvae of *Balanus crenatus* were collected at Pointe Mitis, in the St. Lawrence Estuary, Quebec. Four substrata were sampled and observed using scanning electron microscopy (SEM): shells of *Mytilus edulis*, fronds of *Fucus evanescens*, and laminated panels immersed for periods of 4 and 8 wk. Following this treatment, 23 variables describing the substratum surface, the cover of inert and biological material, and the gregarious response of the larvae were documented for 200 larvae, and compared with 187 control sites uncolonized by larvae. Significant differences of substratum texture, detritus, and diatom abundance were observed between colonized and uncolonized sites, indicating that the selectivity of the larva is related to the heterogeneity of the substratum. On all substrata, the settling site of the larvae was free of detritus and diatoms, clean surfaces ensuring better adhesion for the individual. To rank the variables on which selection occurred, the χ^2 value of a contingency table between each variable and the type of substratum was computed. Variables carrying low χ^2 values represented the most constant larval choices irrespective of the type of substratum. Variables related to the microscopic characteristics of the substratum were more important for larval selection than the variables describing the already established barnacle population.

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Des larves cypris de *Balanus crenatus* ont été récoltées à Pointe Mitis dans l'estuaire du Saint-Laurent (Qué.) sur des valves de *Mytilus edulis*, des frondes de *Fucus evanescens* et des plaques de plastique stratifié immergées durant 4 et 8 sem. Chaque substrat colonisé par les larves a été observé en microscopie électronique à balayage. La surface du substrat, le couvert de matériaux inertes et biologiques de même que le grégarisme des larves ont été décrits par 23 variables sur 200 larves, et ont été comparés aux valeurs observées sur 187 aires témoins non colonisées. Des différences significatives de texture du substrat, d'abondance des détritus et des diatomées ont été observées entre les sites colonisés par les larves et les sites témoins, montrant que les larves sont plus sélectives sur les substrats hétérogènes. Indépendamment de la nature du substrat, les sites sélectionnés par les larves sont exempts de détritus et de diatomées : les surfaces nettes assurent sans doute une meilleure adhésion aux larves. Pour arriver à ordonner les variables selon leur importance pour la fixation des larves, la valeur de χ^2 du tableau de contingence de chaque variable a été calculée en fonction du type de substrat. Ce traitement mathématique a permis de montrer que les variables décrivant les caractéristiques microscopiques du substrat avaient un ordre de priorité plus élevé pour les larves que les variables décrivant les populations de balanes déjà en place sur le substrat.

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NUMEROUS laboratory studies of factors influencing barnacle settlement have shown that site selection is not determined by any single factor, but is dependent upon a combination of variables. Whereas rough (Pyefinch 1948; Barnes 1956) and rugose (Barnes et al. 1951; Crisp and Barnes 1954; Barnes 1955) surfaces are favorable for settlement of barnacle cyprids, the presence of slime or primary film (ZoBell 1939) was reported to influence their settlement (Miller et al. 1948; Daniel 1955; Skerman 1956; Crisp and Ryland 1960; Crisp and Meadows 1963). Such surface films (Crisp 1974) are usually composed of bacteria, diatoms, and detritus (ZoBell 1938). The influence of gregariousness on settlement site selection is shown by cyprid preference for surfaces currently or previously colonized by adults (Knight-Jones and Crisp 1953; Knight-Jones 1953). Recent reviews of the factors influencing barnacle settlement have been provided by Crisp (1974) and Lewis (1978).

The work summarized above indicates the continuing interest in barnacle settlement. However, because most factors influencing settlement were studied individually under laboratory conditions, their relative importance for larval settlement has never been established under natural conditions, where the larvae are subjected simultaneously to the stimuli of texture, surface contour, primary film, gregariousness, etc., in various combinations. In our work, the microscopic and macroscopic characteristics of the sites chosen by 200 larvae were first observed on different substrata using scanning electron microscopy (SEM). Then, the sites selected by the larvae were compared to uncolonized control areas to determine the degree of larval selectivity on substrata of different heterogeneity. Finally, the variables were ranked according to larval preferences, considering that variables bearing the least variation among substrata were the most important for site selection.

Methods

Balanus crenatus Bruguière cyprid larvae were collected in August 1978 on artificial and natural substrata at Pointe Mitis, on the south shore of the St. Lawrence Estuary, Quebec. On August 6, samples were taken from black laminated plastic panels (Conoflex®, Pioneer Plastic, Division of LOF Plastic Inc.) which had been immersed 5 m below mean low tide level (chart datum) for periods of 4 and 8 wk, respectively. At 5 m, a period of 8–10 wk was necessary for the development of a significant surface film in the study area (Hudon and Bourget 1981). On August 16, specimens of *Fucus evanescens* Agardh and *Mytilus edulis* L. supporting numerous cyprids were collected from the sublittoral fringe. This 10-d difference in sampling was due to the delayed presence of settling cyprids in shallower areas.

After cutting the samples down to a convenient size ($1-10$ cm^2, depending on the substratum), portions of substratum supporting the larvae were fixed in 2.5% phosphate-buffered (pH = 7.2) glutaraldehyde for 2 h. They were then dehydrated in graded ethanol solutions (25, 50, 75%) and stored in 75% ethanol. Prior to SEM observation, the samples were transferred to absolute ethanol for 5 min, freeze-dried, mounted on aluminum stubs, and coated with evaporated gold–palladium. The cyprids, their site of settlement, and uncolonized control areas were then observed with a scanning electron microscope operating at a gun potential of 20 kV and a beam angle perpendicular to the sampled surface.

The common occurrence of barnacles on all kinds of solid surfaces could indicate that settlement is more related to accessory characteristics of the surface than to the nature of the substratum itself. Indeed, the settlement of barnacle cyprids on *Mytilus* valves, *Fucus* fronds, and various other natural and artificial substrata is frequently observed (Crisp 1974).

Of a total collection of 200 cyprid larvae, 80 were on *M. edulis*, 56 on *F. evanescens*, and 64 on the two laminated plastic panels which had been immersed for 4 and 8 wk. On the same samples, 187 uncolonized sites were observed, distributed as follows: 82 on *M. edulis*, 45 on *F. evanescens*, and 30 on each type of artificial substratum. Each larva was identified and 23 variables were documented to describe the colonized sites. Uncolonized sites were documented with a subset of 11 variables. The variables (Table 1) were divided into two major categories: one consisting of the basic substratum texture and cover of inert and biological material, the other including the existing population of barnacles as well as the distance maintained between the settled larvae and other individuals. With the description of substratum texture and detrital cover we tried to ascertain the influence of the inherent qualities of the substrata and/or of deposited alien material. The biological material in front of the cyprid was identified to the genus level to detect a possible indicator species (Strathmann and Branscomb 1979). Quantification of biological material also allowed us to examine the need for critical bacterial or diatom densities. For this, a standard enlargement of 500×, defining a 40 000 μm^2 surface, was used at all sites to evaluate the microscopic variables, except for bacteria, which were counted on a 2000× close-up of the same site (surface 1000 μm^2). The neighboring population of barnacles was examined to evaluate settling preference in relation to the size of the individuals. The evaluation of barnacle populations on the samples was made with a dissecting microscope. Measurements of the distance to the nearest neighbor were carried out from scanning electron micrographs, the largest distance to be measured (~3 mm) being defined by the width of the SEM field at 30×.

The intensive exploration of the substratum carried out by the larva prior to its settlement (Knight-Jones and Crisp 1953; Crisp 1961, 1974) provides a first hint that the settling site is the result of a definite choice among numerous alternatives. Therefore, uncolonized sites were determined using coordinates selected with the help of a random number generator. Because some regions of the substrata were never colonized by the larvae (e.g. anterior portion of the shell of mussels), the uncolonized sites examined were all located within the limits determined by barnacles already settled on the surface. This procedure ensured that the unchosen alternative sites had not remained uncolonized because of their nonaccessibility. Statistically, the probability of rejecting the null hypothesis that there was no difference between colonized and uncolonized sites was thus reduced. On the other hand, the sites chosen by the larvae could not be directly examined, as the larvae use their first set of antennae, located underneath the animal, to attach themselves (Darwin 1851; Nott and Foster 1969). It

TABLE 1. List of the variables observed for 200 larvae settled on four types of substrata.

Variable measured	Units	Objectives
Microscopic environment		
A. Basic substrate appearance		
Periostracum[a]	Coded: (0) detached or removed to (5) intact	To evaluate the influence of the nature of the substratum itself and the type of relief available for larval choice
Fucus surface[a]	Coded: (0) intact to (5) damaged	
Texture of substratum[a]	Coded: (0) nonvisible to (5) clearly visible	
Holes[a]	Coded: (0) absent to (5) abundant	
Position in relation to grooves	Coded: (0) out to (1) in	
B. Cover of inert material		
Silt[a]	Coded: (0) absent to (5) abundant	To evaluate the influence of inert material on the site of settlement
Abundance of detritus[a]	Coded: (0) absent to (5) abundant	
Texture of detritus[a]	Coded: (0) fine to (5) coarse	
Distribution of detritus[a]	Coded: (0) uniform to (5) aggregated	
Cover of detritus	%	
C. Cover of biological material		
Bacteria/1000 μm^2	number	To evaluate the influence of the cover of biological material, detect attraction toward a possible "indicator species," and assess the importance of primary film in general
Cocconeis spp.[a]/40 000 μm^2	"	
Synedra spp.[a]/40 000 μm^2	"	
Fragilaria spp.[a]/40 000 μm^2	"	
Macroscopic environment: gregarious response		
A. Population of barnacles on the sample		
Size of the sample	cm^2	To measure the influence of density and detect large-scale gregarious interactions of larvae with different size-groups and/or densities
Larvae[b]	Number	
Newly metamorphosed individuals[c]	"	
Adults < 3 mm	"	
Adults > 3 mm	"	
Cover of calcareous bases	%	
B. Individual interactions		
Distance to the nearest larva	μm	To evaluate small-scale gregarious response and/or the spacing response of the individuals
Distance to the nearest adult	μm	
Position relative to calcareous bases	Coded: (0) not on a calcareous base to (4) entirely on	

[a]Variables quantified on both colonized and uncolonized sites.
[b]Larva defined as an unmetamorphosed cyprid.
[c]Newly metamorphosed defined as an uncalcified metamorphosed barnacle.

was thus decided to examine the closest possible area within the operating radius of this sense organ, which is the area located in front of the anterior portion of the attached cyprid.

To determine whether a choice was made for certain values of a given variable, the site located immediately in front of each larva was compared to randomly selected uncolonized sites, using the Mann–Whitney U test. This test was also used to detect the occurrence of a significant difference in the mean settlement distance of larvae from adults and from other larvae as nearest neighbors.

The second phase of the analysis consisted in the ranking of the variables according to the constancy of larval choice, by comparing the frequency of the various classes of each variable among substrata. As some variables were quantitative and others were coded, the quantitative variables had to be recoded into discrete states to homogenize these comparisons and to make it possible to rank the variables following Legendre and Legendre (1983). The method can be summarized as follows: the continuous discriminant variable (D) to be coded was first replaced by rank orders and then divided into two states, trying in turn all possible partitions. For each alternative, a contingency table of the states of D by the states of the reference classification variable (C, the type of substratum) was drawn, and the dependence between the reference variable (C) and the discriminant variable (D) was measured through Wilks' (1935) likelihood ratio statistic:

$$\chi^2 = 2 \sum_{\substack{\text{All cells}\\ \text{of table}}} O \ln (O/E), \text{ for all } O, E > 0$$

whose value is asymptotically distributed as χ^2 when the total number of observations (p) is large. The statistic uses the natural logarithm (ln) of the ratio of the observed frequencies (O) to the expected values (E). Expected values are computed, as usual, as:

$$E = \frac{\text{total of the row} \times \text{total of the column}}{p}$$

and allows one to test the null hypothesis of independence between C and D.

The best partition into two states is attained with the highest values of χ^2, as it indicates the lowest probability of independance between C and D. Then, holding the first partition fixed, a second partition is added and the χ^2 values are again computed for all the possible three-state partitions of the discriminant variable. The optimal number of partitions of the variable into n classes is attained when the probability associated with χ^2 is lowest.

The probability associated with Wilks' likelihood ratio statistic (χ^2) also allows for the computation of the amount of information (B) shared by the classification of C and D, through Kullback's (1959) relation:

$$B = \chi^2/2p.$$

To obtain a value standardized between 0 and 1, the following ratio was then computed for each variable, in the same way as the "evenness" component of diversity (Lloyd and Ghelardi 1964):

$$R = B/H_{\max} \text{ or } R = B/\ln n = \chi^2/2p \ln n.$$

The maximum information ($H_{\max}$) is obtained, for any given number of classes (n) in the coded variable, when the probabilities of all the states are equal. Both B and $H_{\max}$ are calculated from the same logarithm base (in this case, ln), thus accounting for the "standardizing" effect.

The χ^2 and R values will be used to rank the variables as to their importance in the settling decision process of the larvae.

Results

Microscopic Variables

To describe accurately the larval settlement area, it was necessary to evaluate its textural, detrital, and biological uniformity (Table 2). For all substrata, common trends were observed in the settling area selected by the larvae, allowing us to describe the "typical" settlement site. The typical settlement site had an uneven surface, due to the visible presence of mussel growth ridges, algal cells, or plastic striation. The periostracum of *M. edulis* and the cells of *F. evanescens* were usually intact, as was the artificial substratum. On natural substrata, half the larvae were found in grooves, usually parallel to one another. The settling sites were occasionally covered by a uniform layer of small inert particles. Large deposits of silt, or a thick cover of agglomerated organic particles, were very seldom observed.

The biological material covering the typical settlement site comprised many forms of bacteria (cocci, rods, filaments) in abundance varying from 0 to more than 500 cells/1000 μm^2 regardless of the type of substratum. The mean cell densities varied from 186 per 1000 μm^2 on *M. edulis* to 411 cells/1000 μm^2 on the artificial substratum immersed for 4 wk (Table 2). Diatoms were occasionally present on the settlement site, in numbers from 0 to 80 cells on the 40 000 μm^2 area located in front of the anterior portion of the larva. Diatoms were observed in low densities on the colonized sites on natural substrata, but were absent from those observed on plastic.

Although the constancy of the characteristics of the typical settlement site represents evidence for a choice effect, it is necessary to compare the qualities of the selected sites to those of the available, but uncolonized sites. In this respect, a choice effect is indicated for a certain value of any given variable if it is found among colonized sites in a significantly higher (preference) or lower (avoidance) proportion than in uncolonized sites. However, because the larvae cannot possibly occupy all the potentially favorable sites available in an area, the uncolonized sites should include both suitable and unsuitable sites, resulting in a lesser contrast and a lower significance when comparing colonized and uncolonized sites. Comparisons between colonized and uncolonized sites were made using the Mann–Whitney U statistic (Table 2). The general heterogeneity is reflected by the number and significance level of the differences observed for each type of substratum, the highest being achieved by *M. edulis*, and the lowest by the artificial substratum immersed for 4 wk.

From all the substrata investigated, *M. edulis* was the one offering the greatest diversity of primary films (bacteria, epiphytes, silt, and detritus). The larvae generally settled in the widest part of the valve. Among the sites available on a valve, one may find areas densely covered with detritus, mud, or diatoms as well as areas devoid of such primary film. Larvae never settled on large amounts of coarse, agglomerated detritus, and this choice was significant, as shown by the differences for these variables when colonized and

TABLE 2. Adjusted median (for coded variable groups A and B) or mean and standard error (SE, for variable group C) of variables on 40 000 μm^2 areas located either in front of the anterior portion of 200 larvae (colonized sites) or on 187 randomly selected uncolonized sites. The comparison between the values observed for each variable in the frontal area (colonized sites) and randomly selected uncolonized sites was made using the Mann–Whitney U statistic. See Table 1 for the meaning of the coded values. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, ns, not significant.

	Natural substrata						Artificial substrata					
	Mytilus edulis			*Fucus evanescens*			8 wk			4 wk		
	Colonized sites	*P*	Non-colonized sites	Colonized sites	*P*	Non-colonized sites	Colonized sites	*P*	Non-colonized sites	Colonized sites	*P*	Non-colonized sites
Number of sites	80		82	56		45	35		30	29		30
Substratum texture												
Texture of substratum	3.23	ns	2.13		Not observed		2.57	*	3.70	4.87	ns	4.96
Periostracum	4.94	ns	4.80		Not observed			Not observed			Not observed	
Fucus surface		Not observed		2.10	***	4.25		Not observed			Not observed	
Holes		Not observed		0.28	*	1.57	0.15	ns	0.10	0.04	***	0.61
B. Cover of inert material												
Silt	0.03	**	0.18		Not observed			Not observed			Not observed	
Abundance of detritus	1.35	**	2.11	1.37	*	1.96	1.89	ns	1.38	1.10	ns	1.06
Texture of detritus	1.17	***	2.25	1.10	***	2.40	2.42	ns	2.39	0.31	ns	0.89
Distribution of detritus	0.86	***	2.06	1.13	***	2.55	1.94	ns	2.50	0.31	*	0.98
C. Cover of biological material												
Bacteria[a]/1000 μm^2	185.81		Not observed	214.53		Not observed	308.19		Not observed	411.13		Not observed
	(25.17)			(33.26)			(58.64)			(66.40)		
Cocconeis spp./40 000 μm^2	3.57	ns	5.46	0.95	*	3.96	0.86	ns	8.17	0	b	0.13
	(1.52)		(1.41)	(0.30)		(0.86)	(0.41)		(3.36)	(0)		(0.10)
Synedra spp./40 000 μm^2	3.67	*	15.76	0.34	**	2.11	0	b	0	0	b	0.03
	(1.19)		(3.77)	(0.10)		(0.57)	(0)		(0)	(0)		(0.03)
Fragilaria spp./40 000 μm^2	0.65	ns	0.81	0	b	0.24	0	b	0.07	0	b	0
	(0.35)		(0.27)	(0)		(0.22)	(0)		(0.05)	(0)		(0)

[a]No comparison was made for bacterial densities.
[b]Mann–Whitney test cannot be performed on empty groups.

TABLE 3. Mean and standard error values for six variables describing the populations of barnacles already colonizing the four types of substrata.

	Natural substrata				Artificial substrata			
	Mytilus edulis		*Fucus evanescens*		8 wk		4 wk	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Dimensions (cm^2)	5.53	0.40	0.85	0.10	4.0	0	4.0	0
Number of larvae	2.65	0.41	1.86	0.33	6.0	2.5	3.38	0.63
Number of newly metamorphosed larvae	9.97	2.05	4.67	1.09	18.67	5.19	11.88	1.83
Number of adults < 3 mm	2.74	0.87	1.81	0.58	2.83	1.45	4.88	3.18
Number of adults > 3 mm	0.29	0.23	0		0		0	
% cover of calcareous bases[a]	7.68	0.06	3.31	0.07	71.39	1.5	0	

[a]The % cover of calcareous bases was computed after data were transformed using angular (arcsin) transformation.

uncolonized sites were compared (Table 2). The primary films available on *Fucus* were less diversified, with occasional patches of detritus and epiphytes, all of which also appeared to be avoided by the larvae. On the two natural substrata, the larvae had a tendency to colonize surfaces with low densities of diatoms. Greater homogeneity of sites on artificial substrata is shown by a lower level of significance of differences observed between colonized and uncolonized sites. In summary, for the four substrata, the actual location of the larvae was definitely the result of a choice among different alternative conditions, particularly the abundance of detritus, epiphytes, and the texture of the surface.

MACROSCOPIC VARIABLES: GREGARIOUS RESPONSE

The organisms already occupying the substratum may influence larval settlement in two possible ways. On a large scale, differences in the size and age distributions within existing barnacle populations could produce different gregarious responses, showing possible preferences of the larvae for certain densities or age-groups. On a small scale, larval response to its own species may be reflected in the way individuals settle on the substratum, maintaining a certain distance from each other to avoid overcrowding (Crisp 1961; Knight-Jones and Moyse 1961). Again, the size of the neighboring individuals could influence the distance at which the larva will settle.

The mean values of the six variables describing the barnacle population observed (Table 3) show that the four substrata supported vastly different established macrofaunas. As the samples of natural substrata were arbitrarily cut down to a suitable size, between-substrata comparisons of barnacle densities must be made with caution. In this context, three general observations can be made: (1) newly metamorphosed individuals form the most numerous group on all substrata; (2) larger adults (>3 mm) are only present on *M. edulis*; and (3) the cover of calcareous bases is important only on the artificial substratum immersed for 8 wk. The comparison between panels of similar size immersed for 4 and 8 wk reveals a larger number of dislodged adults (as indicated by the calcareous bases), of newly metamorphosed individuals and of larvae on the panel immersed for the longer period.

The mean values of distance to the nearest adult and to the nearest unmetamorphosed larva (Clarke and Evans 1954) (Table 4) were not significantly different on any substratum, indicating a similar spacing response toward adults and larvae. However, on mussels, there was a tendency for larvae to settle closer to one another and to adults than on other substrata. This apparent difference may have resulted from the initial degree of crowding on the substrata. The examination of the proportion of larvae settled within a radius of 3.0 mm to the nearest larva or adult shows a generally greater proportion of larvae in the neighborhood of another larva than of an adult. The highest proportion of larval settlement close to another larva (74%) was observed on the artificial substratum immersed for 4 wk. Larvae settled in the neighborhood of adults were seen most often on *M. edulis*, on which the adult size-group was best represented. Similarly, the percentage of larvae settled on a calcareous base seems roughly proportional to the surface covered by these remains on each type of substratum.

Overall Analysis and Discussion

Our observations describe the settling sites of cyprids and agree in general with the results of previous laboratory experiments. So far, however, the relative importance of each variable in the choice of the settlement site of the larvae remains unknown. To avoid the distortion due to the characteristics not common to all substrata (e.g. periostracum), data referring specifically to one type of substratum were not included in the overall analysis. Also, variables observed on less than 175 larvae (e.g. distance to the nearest neighbor) were not included in the calculation of the χ^2 statistics. As variables were not all in the same form, they were all used in their coded form (see Methods) for comparison between substrata. The use of different substrata provides a good way to emphasize the factors subjected to variation, as this single

TABLE 4. Mean and standard error (SE) values of distance to the nearest neighbor (larva and adult) for larvae observed on four different substrata.

Substrata	Natural substrata		Artificial substrata	
Total number of individuals (number measured)[a]	*Mytilus edulis* $n = 80$ (37)	*Fucus evanescens* $n = 56$ (55)	8 wk $n = 35$ (32)	4 wk $n = 29$ (25)
Distance to the nearest larva (μm) (SE)	720.9 (72.7)	1032.5 (133.5)	1168.2 (90.1)	1213.0 (122.1)
Number of individuals	$n = 24$	$n = 35$	$n = 22$	$n = 23$
(% of number measured)	(65%)	(64%)	(69%)	(92%)
Distance to the nearest adult (μm) (SE)	804.2 (102.2)	1100.0 (153.4)	815.0 (184.4)	1700.0 (364.0)
Number of individuals	$n = 23$	$n = 20$	$n = 10$	$n = 8$
(% of number measured)	(89%)	(36%)	(31%)	(32%)
Number of larvae settled on calcareous bases	6	0	22	0

[a]Number of individuals settled within a 3.0-mm radius of the next individual.

basic difference can include an array of associated characteristics while staying within the range of natural conditions. This aspect is reinforced by the availability of vastly different types of primary film in a small region of natural substratum. Futhermore, one can find a strong positive relationship in the shared information between the type of substratum and all other variables shown by a very highly significant value of B (equal to $\chi^2/2p$). Because all four substrata are colonized by larvae, the larval choice will express itself in the characteristics associated with the settling site.

In fact, the larvae will show their preferences in the constancy of their choices, which can be asserted in three ways: the modal values, the evenness ratio (R), and the likelihood ratio statistic (χ^2) of the variables. The number of classes of the coded variables was initially selected to cover the whole range of variations occurring on the substrata (Table 1). The initial observations on the larvae showed that only certain values of scales, either low (e.g. detritus) or high (e.g. texture) (Table 2), were found significantly more often near the larvae than on uncolonized sites, thus indicating a choice. This result is confirmed by low values of the R ratio (Table 5), which can be used as a weighting factor of the likelihood ratio statistic (χ^2). This latter value is expected to bring up the differences solely as a result of the type of substratum, while the variables remaining constant despite the substratum nature, and showing the lowest χ^2 values, indicate the most important larval preferences.

The results of the analysis (Table 5) confirm the observations previously summarized. The variables that allowed the largest fluctuations among substrata, and thus the least important to the larvae, are related to the structure of barnacle population colonizing the samples. The highest level of relationship is due to the percentage cover of barnacle calcareous bases, followed closely by the number of newly metamorphosed individuals. Thus as a whole, this group of macroscopic characteristics bore high values of the χ^2 statistic. The results indicate that although the larval choice is positively influenced by the presence of individuals of its own species, it is not strongly related to any population size-composition or size-group. The larval attraction toward its own species would thus be restricted to the basic chemosensitive reaction to an active agent (Crisp and Meadows 1963; Gabbott and Larman 1971), whatever its exact "biological" form is.

TABLE 5. Variables in ranked order of likelihood ratio statistic (χ^2) indicating their relationship to the type of substratum. High values of the coefficient (χ^2) indicate variables showing large fluctuations from one substratum to another. Evenness ratio (R) has the same meaning as χ^2 but is expressed in terms of the information theory.

Variable	χ^2	R
% cover of calcareous bases	177.81	0.32
Position relative to grooves	120.55	0.15
No. of newly metamorphosed individuals	108.44	0.08
No. of larvae	72.52	0.13
No. of barnacles <3 mm	71.07	0.05
Texture of the substratum	56.52	0.01
No. of *Synedra* spp. cells	42.81	0.04
% cover of detritus	35.78	0.07
Distribution of detritus	34.01	0.005
Abundance of detritus	33.28	0.005
No. of *Cocconeis* spp. cells	23.96	0.05
Silt	20.01	0.002

The high χ^2 value for the position of larvae relative to grooves emphasizes the inherent differences of contour between natural and artificial substrata. The contingency due to the selective effect of presence/absence of strong relief (grooves, pits, etc.), combined with the fact that only half the larvae found on natural substrata were actually settled in grooves, suggests that the larvae were probably influenced by other factors, such as the detrital cover in the available grooves.

Texture is another important variable used to distinguish the substrata. Although the four substrata are generally rough (Table 2), some differences in the degree of roughness can be found. Larvae would therefore seek a certain range of roughness, varying from medium to high values.

At the bottom of the list, the factors with both low χ^2 and R values indicate the most constant choices of the larvae.

These variables are the low abundance of detritus, of *Cocconeis* spp. cells, and silt. A correlation between algal abundance and larval settlement was demonstrated for intertidal barnacles by Strathmann et al. (1981). The difference observed for *B. crenatus* may indicate a smaller importance of diatoms as a cue for the survival of a subtidal species. However, the adaptive significance of the choice of an area free of detritus and diatoms is clear. It corresponds to the need for a firm attachment site for the antennules (Walker 1973) and for space required when metamorphosis occurs. Indeed, the forward tilting on the axis of the antennules requires a relatively clean area for an eventual permanent attachment. Later on, as the newly metamorphosed barnacle cements itself more permanently to the substratum (Walker 1973; Walker and Lee 1976), a clean area will ensure strong adhesion, hence a better chance of survival.

As a whole, our results confirm the complexity involved in the selection of a settling site for barnacle larvae. Chemosensitive reaction to an active agent could be responsible for the general attraction towards potentially favorable surfaces. An initial exploration is made by the larva to determine if roughness and surface contour are adequate. Finally, cyprid larvae select an area devoid of detritus and diatoms for their settlement, possibly even using their antennules to brush away detritus and diatoms (Crisp and Meadows 1963) in an active modification of the substratum to suit their needs.

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